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APPLICATION NUMBER: *60/552,892*
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Sir:

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Title: METHOD AND APPARATUS FOR PATCH-CLAMP ANALYSIS

Enclosed are:

[X] 18 pages of the application (including description, claims, abstract and appendix).
[X] 14 sheet(s) of [ ] formal [x] informal drawing(s).
[X] Abstract.
[X] 15 claims.
[X] Small Entity Status is Claimed
[X] Cover Sheet.
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22798

PATENT TRADEMARK OFFICE

Attorney Docket No. 313S-300800US
Client Reference No. B04-030
Express Mail Label No. EL985938696US
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By: Stephen J. LeBlanc

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METHOD AND APPARATUS FOR PATCH-CLAMP ANALYSIS

ABSTRACT OF THE DISCLOSURE

A method and/or system using an integrated patch clamp and methods for construction of the same.

Application Information

Application Type::	Provisional
Subject Matter::	
Suggested Classification::	
Suggested Group Art Unit ::	
CD-ROM or CD-R?::	
Number of CD disks::	
Number of copies of CDs::	
Sequence submission::	
Computer Readable Form (CRF)?::	
Number of copies of CRF::	
Title Line One::	METHOD AND APPARATUS FOR PATCH-
Title Line Two::	CLAMP ANALYSIS
Title Line Three::	
Attorney Docket Number::	313S-300800US
Request for Early Publication?::	
Request for Non—Publication?::	
Suggested Drawing Figure::	
Total Drawing Sheets::	
Small Entity::	YES
Petition included?::	
Petition Type::	
Secrecy Order in Parent Appl.?::	

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City of mailing address::	Orinda
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Country of mailing address::	USA
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Representative Customer Number::	22798

Assignee Information

Assignee Name:: The Regents of the University of California

WHAT IS CLAIMED:

1. A method of detecting one or more characteristics of cell surfaces comprising:
placing one or more cells of interest into an integrated microfluidic patch-clamp array chip providing easy cell trapping; easy optical characterizations; and simple cell loading for multiple single cell analysis.

2. A method of fabricating an integrated patch clamp device comprising:
preparing a mold by making height patterns defining narrow patch channels using deep etching;
adding patterns for wide connection regions;
introducing a settable material into the mold and curing;
detaching the set material from the mole;
placing holes for connection of tubes;
connecting tubes to reservoirs, via said holes, to load cells and/or electrolyte solutions and to apply suction to patch channel.

3. The method of claim 2 further wherein:
said mold is constructed from silicon.

4. The method of claim 2 further wherein:
said mold is constructed from a ceramic.

5. The method of claim 2 further wherein:
said mold is constructed from a metal or metal alloy.

6. The method of claim 2 further wherein:
said mold is formed using surface micromachining techniques.

7. The method of claim 2 further wherein:
said patterns defining the narrow patch channels are formed using deep reactive ion etching; and further patterns are added for wide connection regions using photoresist.

8. The method of claim 2 further wherein:
said moldable material comprises polydimethylsiloxane (PDMS) and a curing agent.

9. The method of claim 2 further comprising:
subsequently bonding a molded device to a thin PDMS layer which was spin cast and then cured onto a glass substrate.

10. A integrated patch-clamp array chip comprising:
a cell loading reservoir for introducing cells and cell carrying liquid;
a location for drawing fluid and cells from the cell reservoir along at least one cell flow path;
at least one patch channel along said cell flow path; and
a suction location for introducing section at said patch clamp.

11. The device according to claim 10 further comprising:
circuitry for measuring electrical characteristics at said patch channel.

12. The device according to claim 10 further comprising:
individually addressable circuitry at each patch channelfor separately determining electrical characteristics at said clamps.

13. The device according to claim 10 further wherein:
patch channels are in a horizontal plane allowing multiplexed parallel patch sites that are only tens of μm apart are possible.

14. The device according to claim 10 further wherein:
patch channels are in a horizontal plane with multiplexed parallel patch sites having a distance between patch sites of between one hundred μm and one thousand μm.

15. The device according to claim 10 further comprising:
microfluidic features to move substances to appropriate positions of said device.

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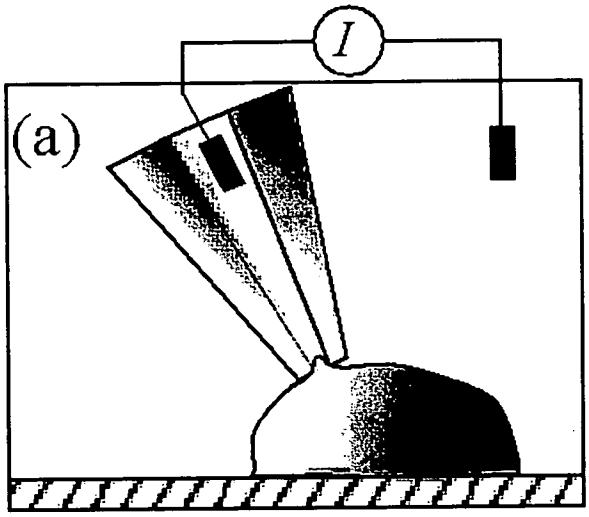

FIG. 1A

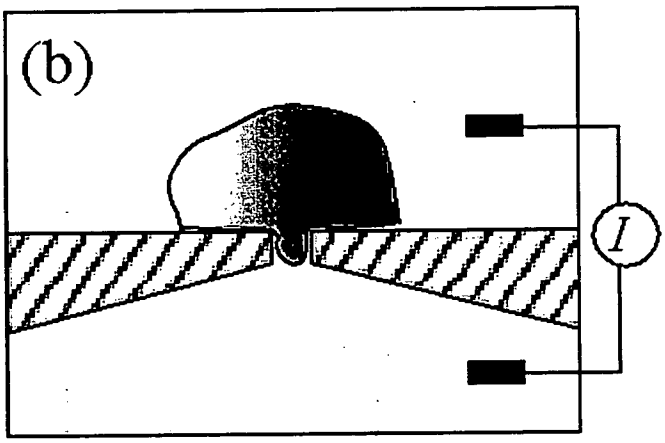

FIG. 1B

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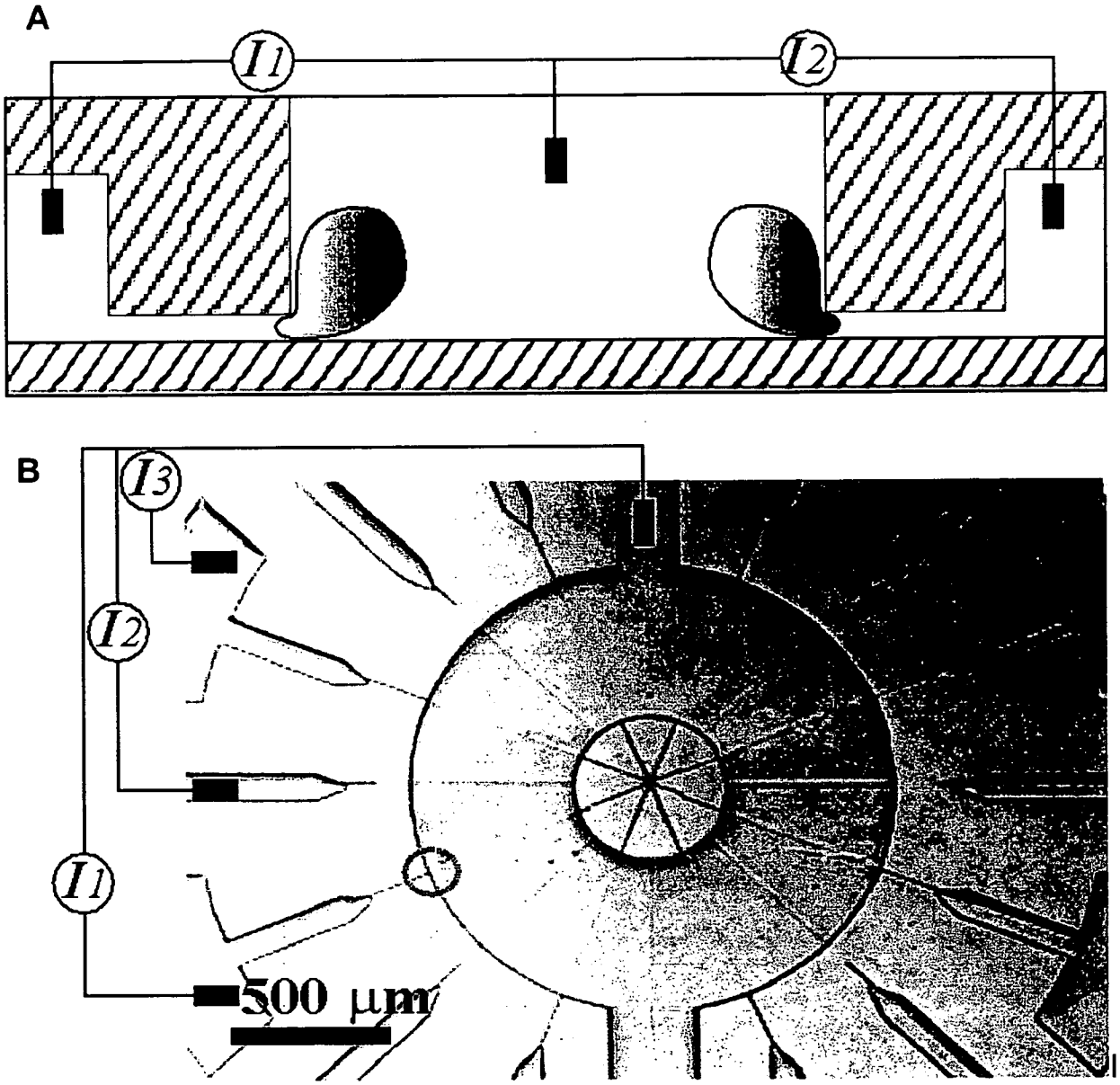

FIG. 2

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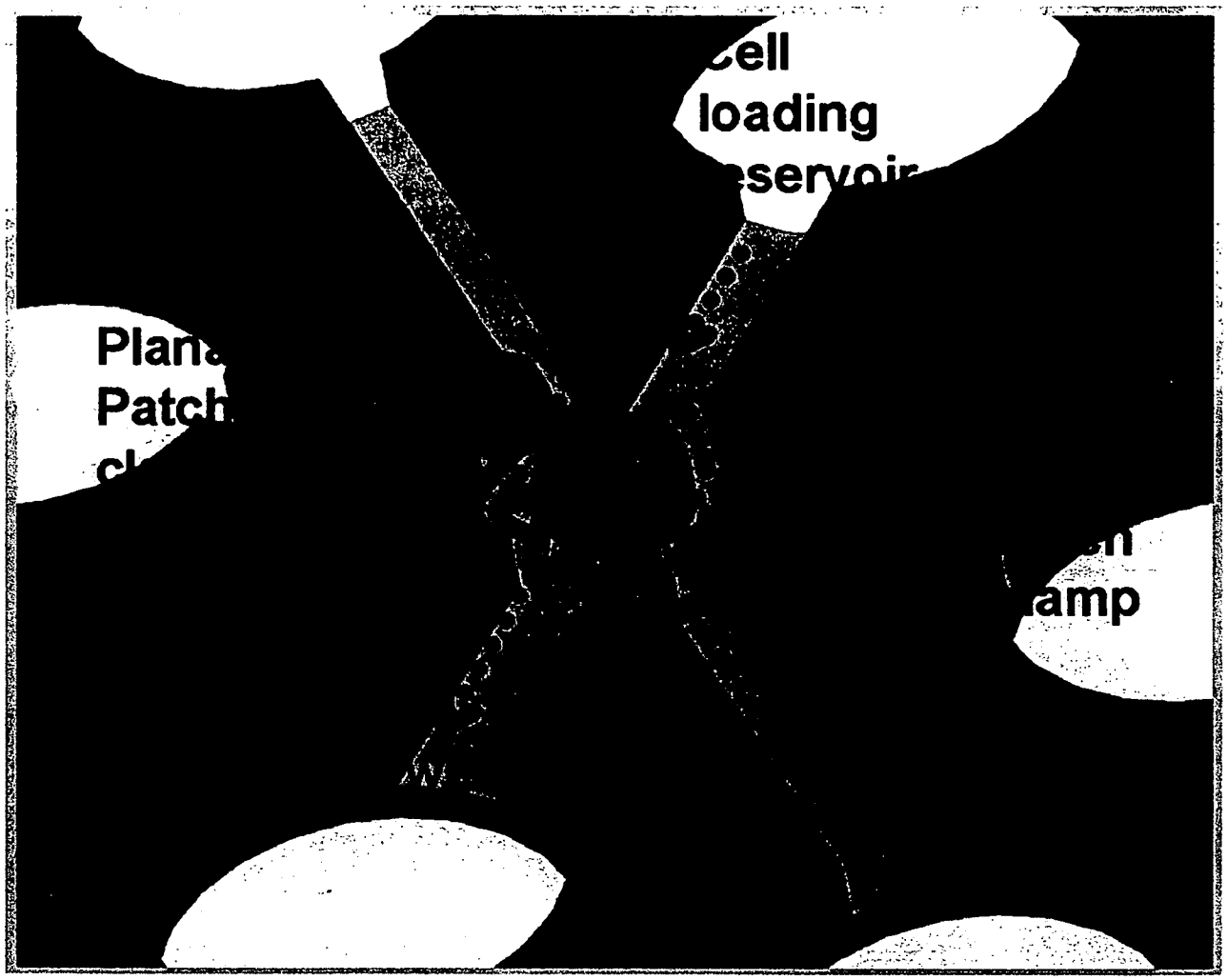

FIG. 3

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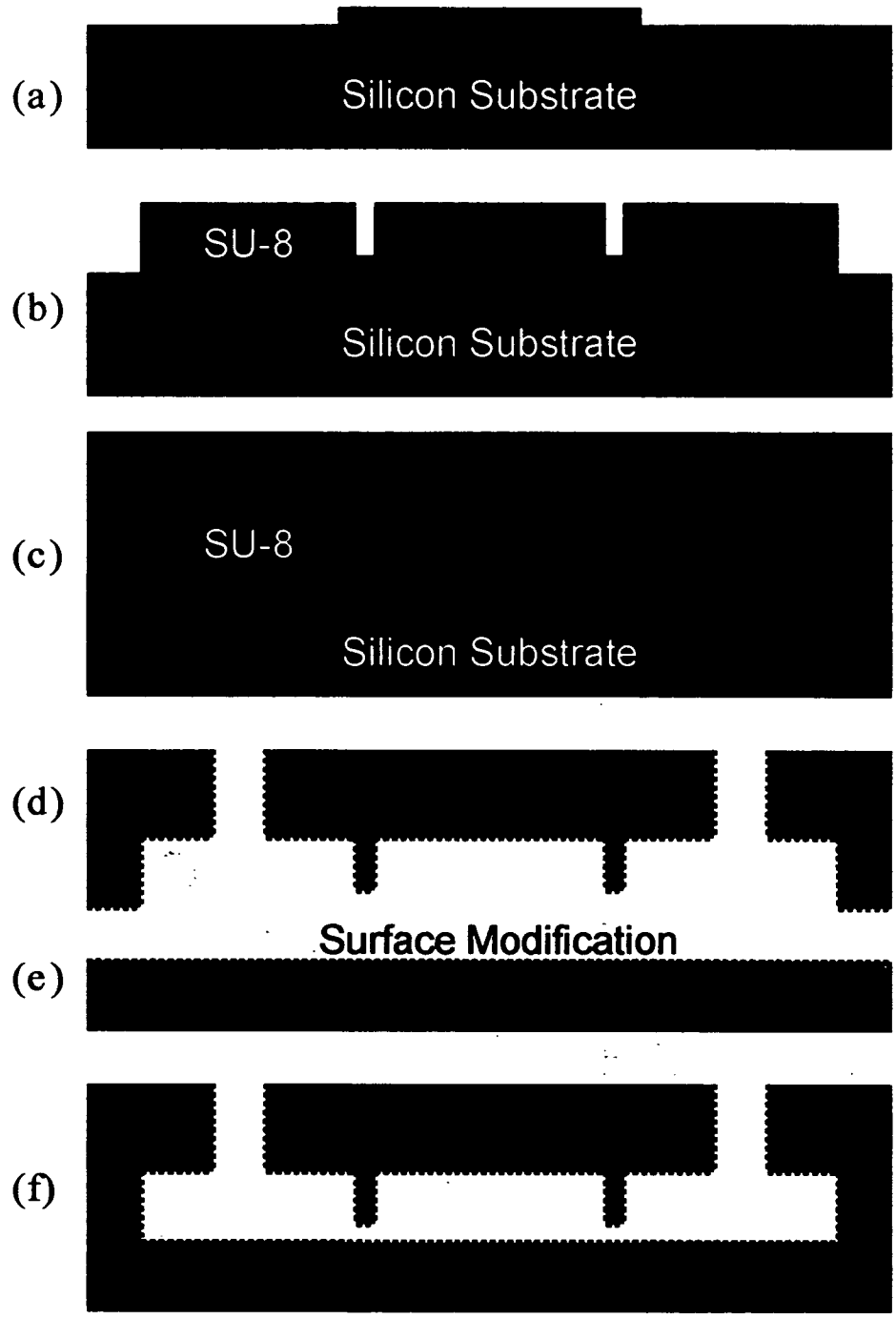

FIG. 4 (PART 1)

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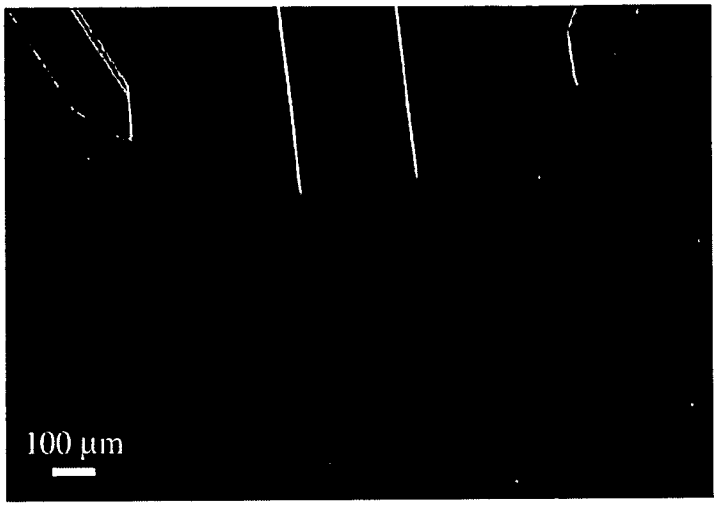

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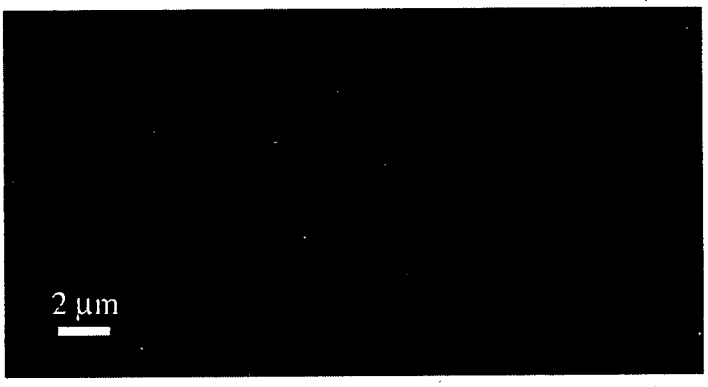

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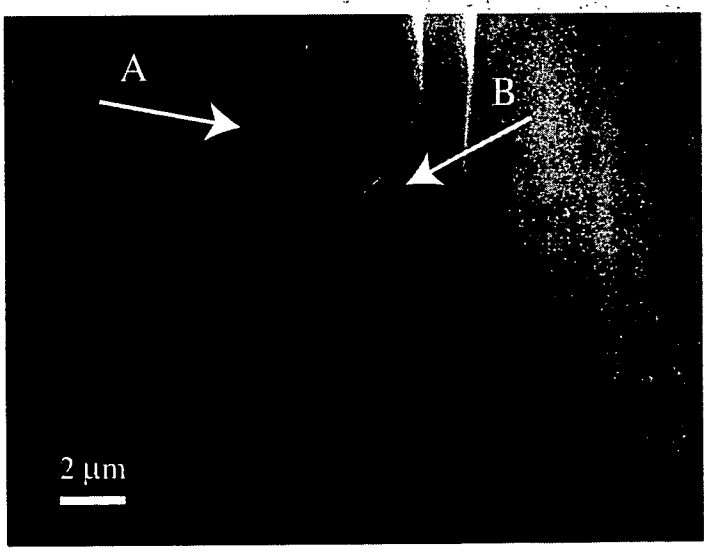

FIG. 4 (PART 2)

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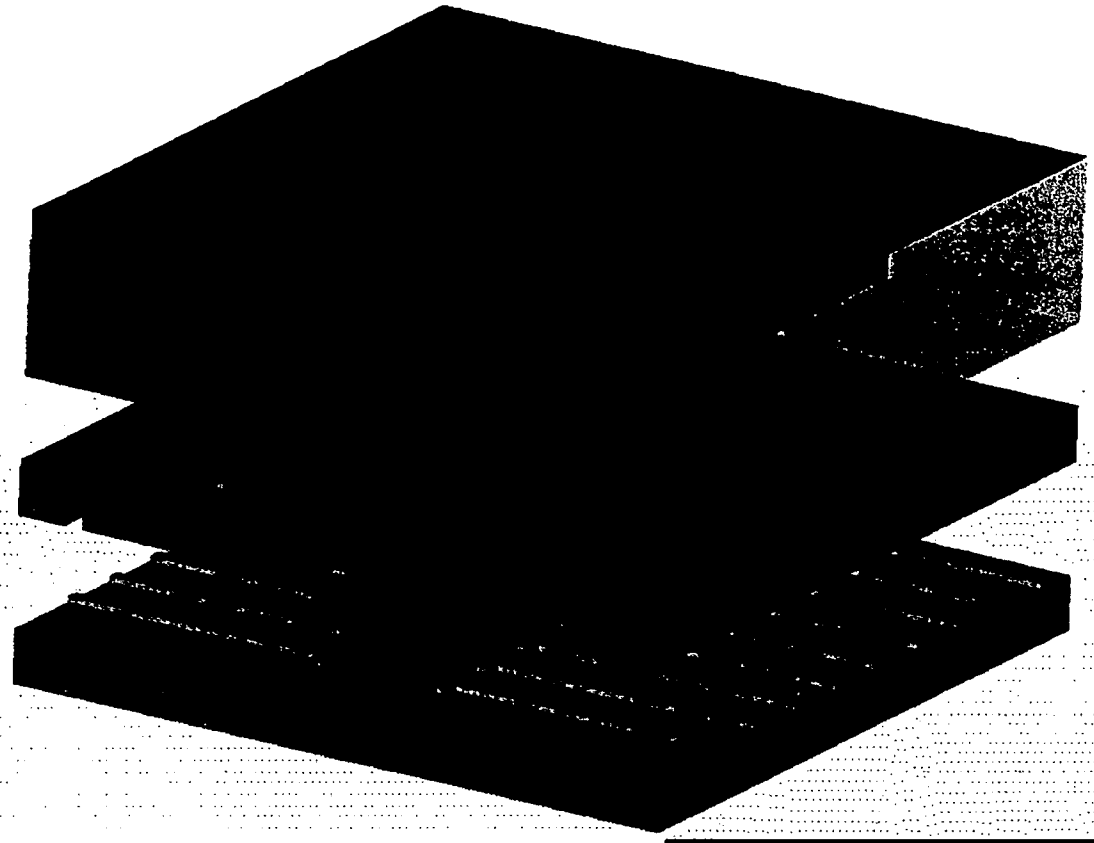

- Sample fluidic channels
- Control fluidic channels
- Integrated reference electrode
- Cells

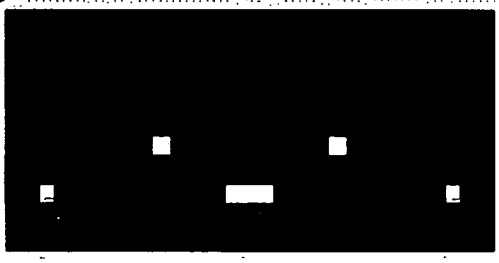

FIG. 5A

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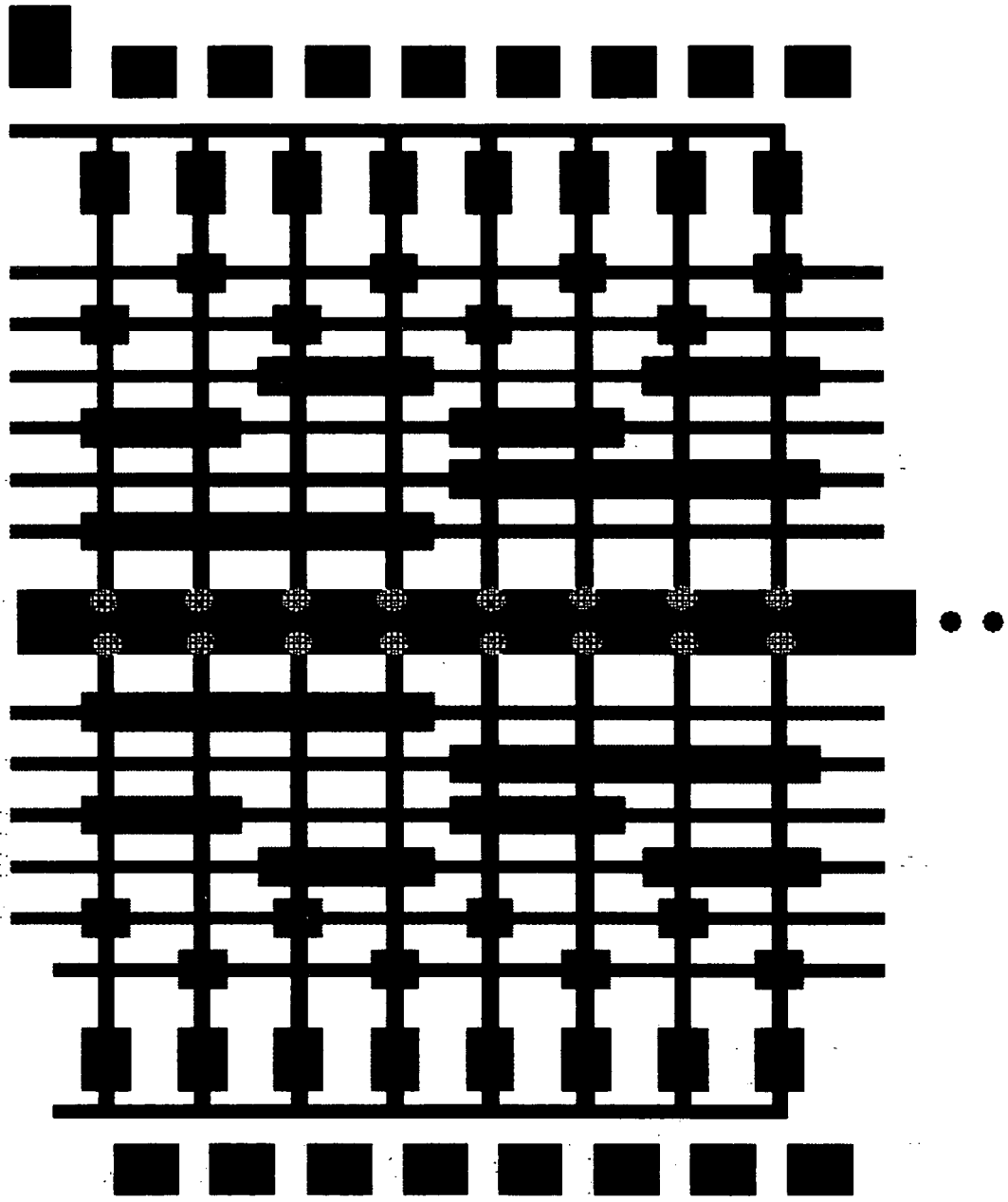

FIG. 5B

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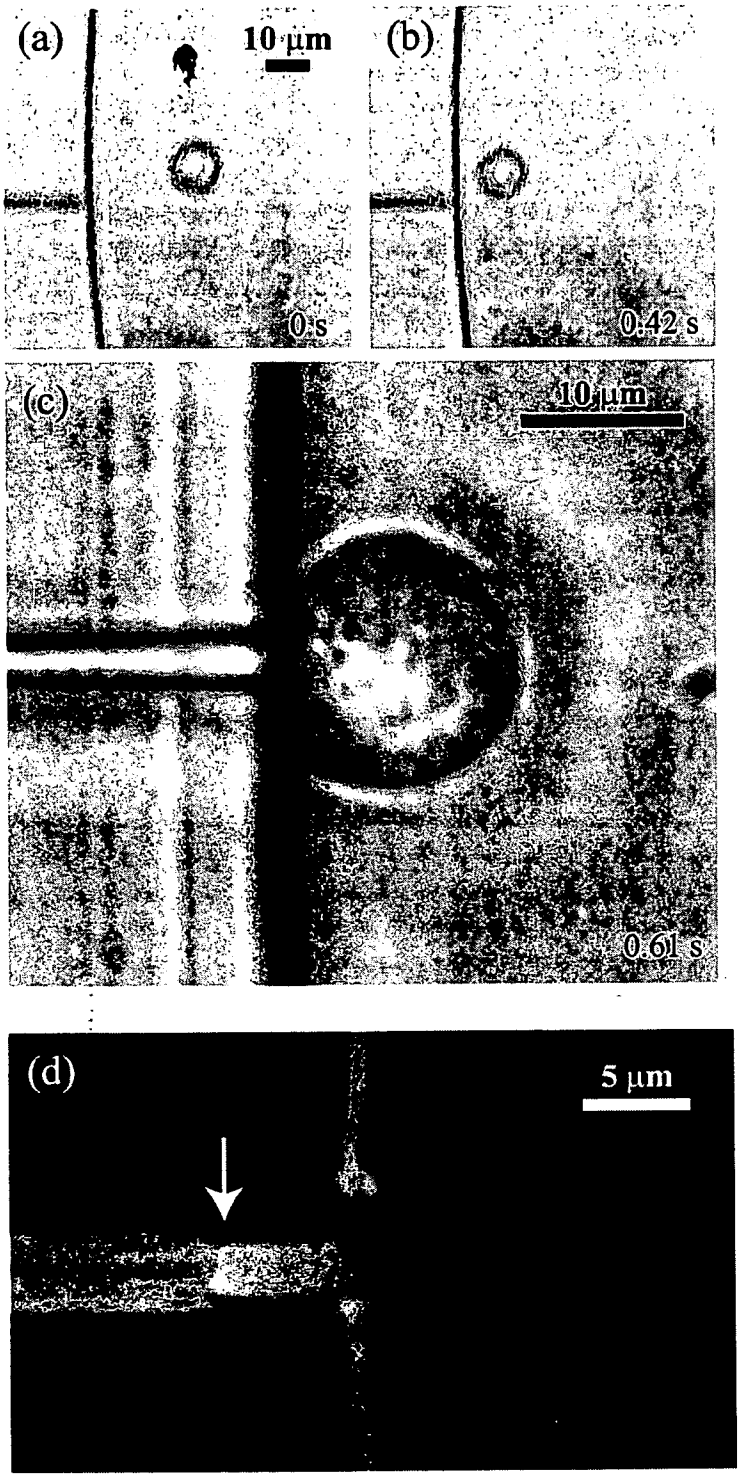

FIG. 6

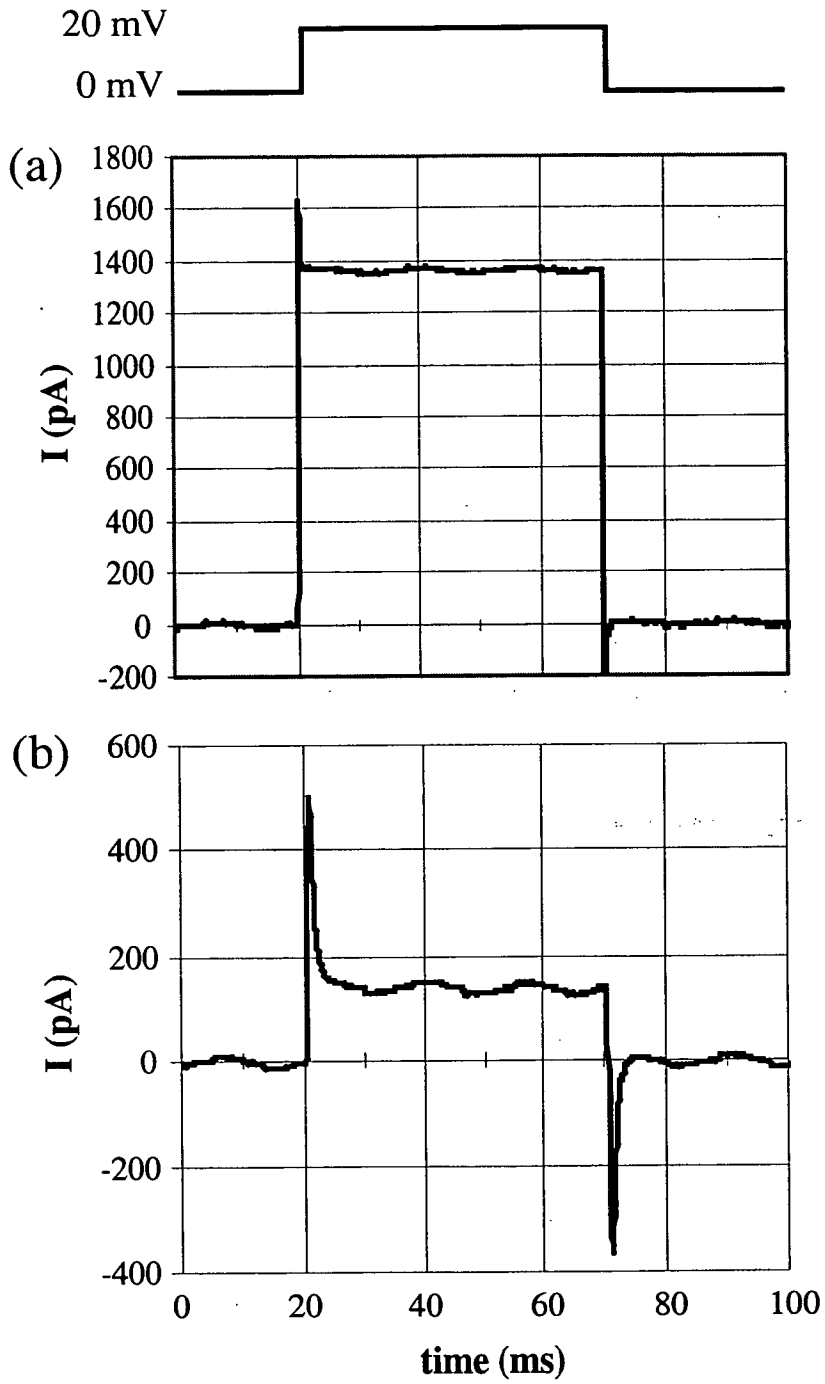
20 mV
0 mV
(a)
1800
1600
1400
1200
1000
800
600
400
200
0
-200
I (pA)
(b)
600
400
200
0
-200
-400
I (pA)
0
20
40
60
80
100
time (ms)

FIG. 7

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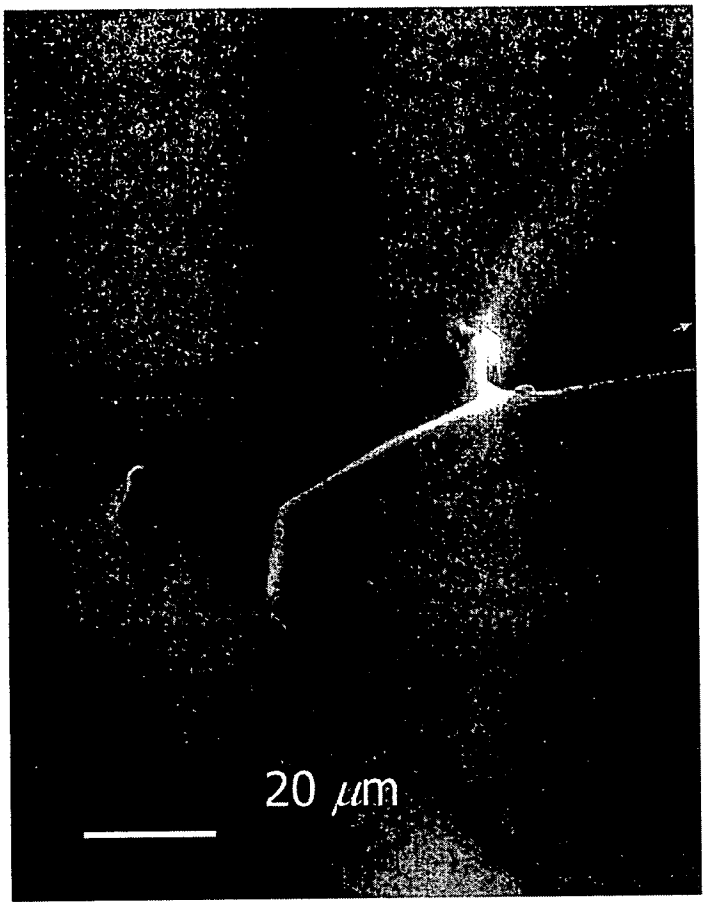
FIG. 8A



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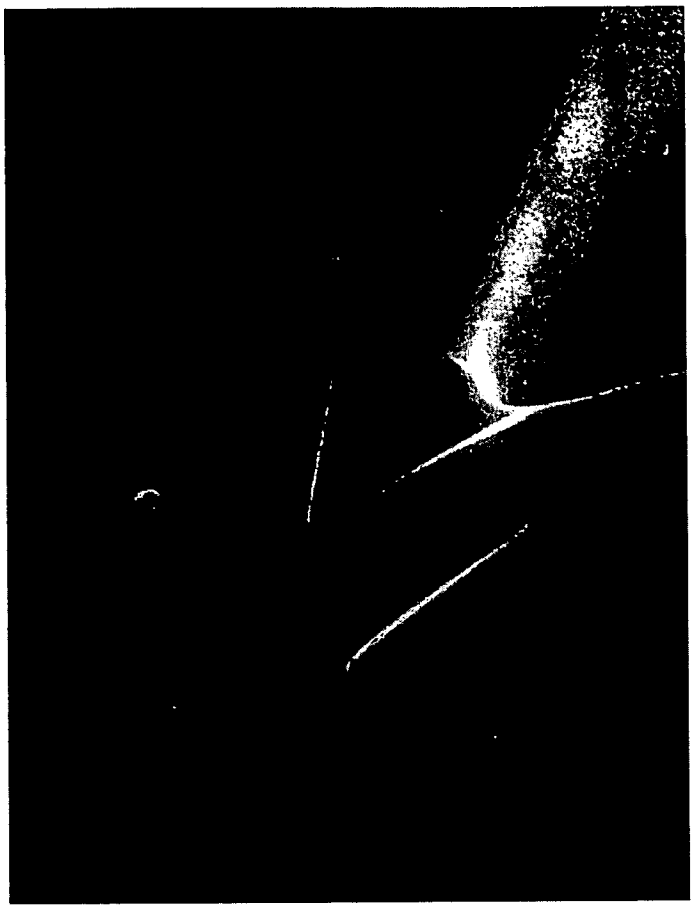

FIG. 8B

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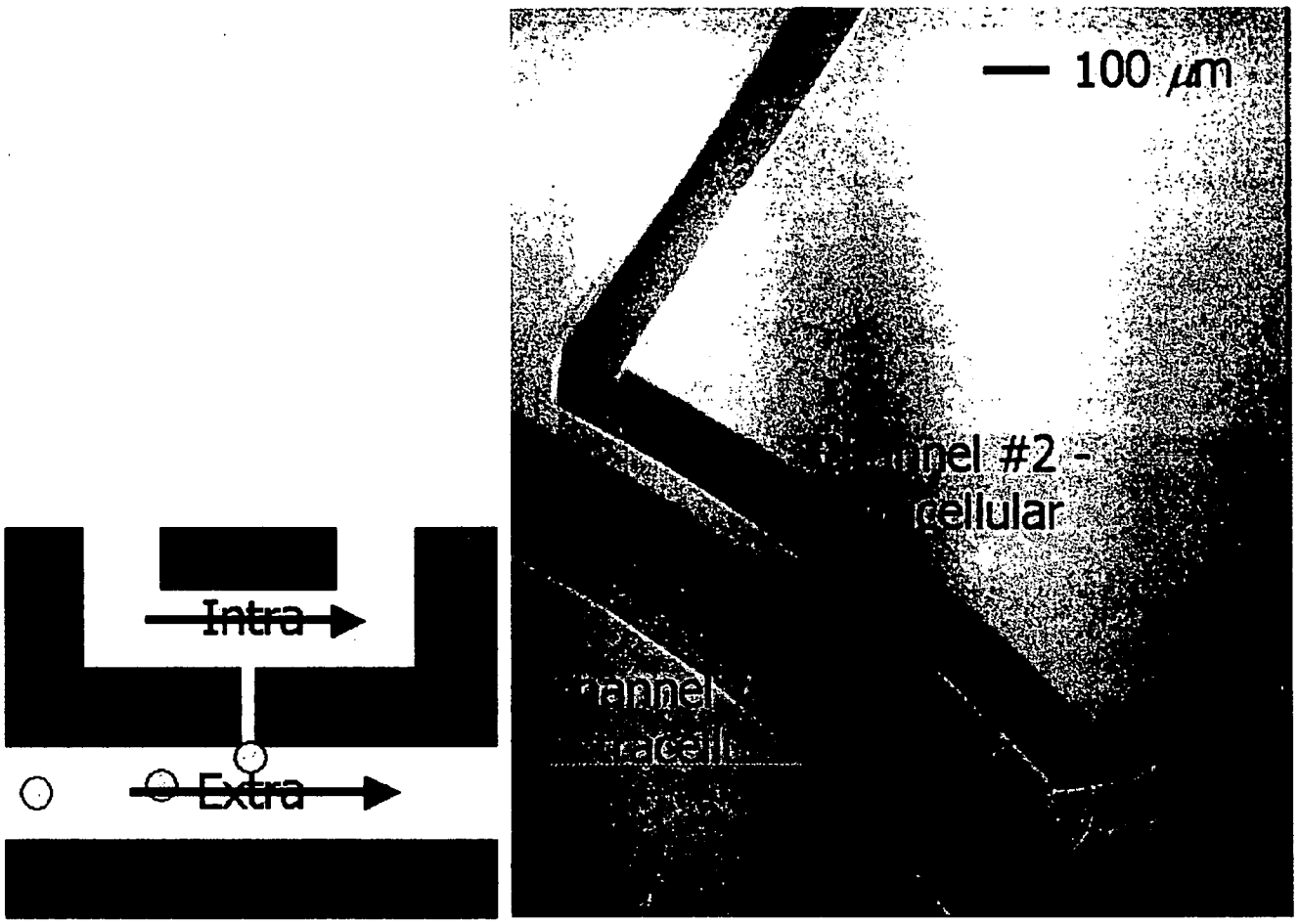

FIG. 9

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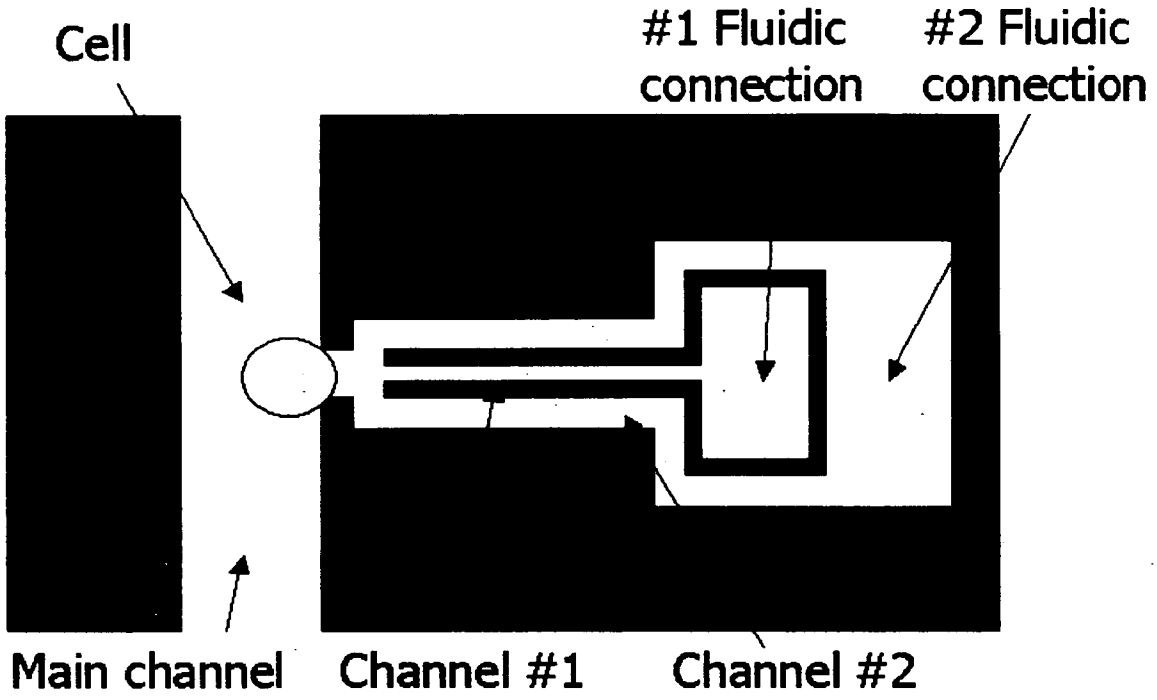

FIG. 10

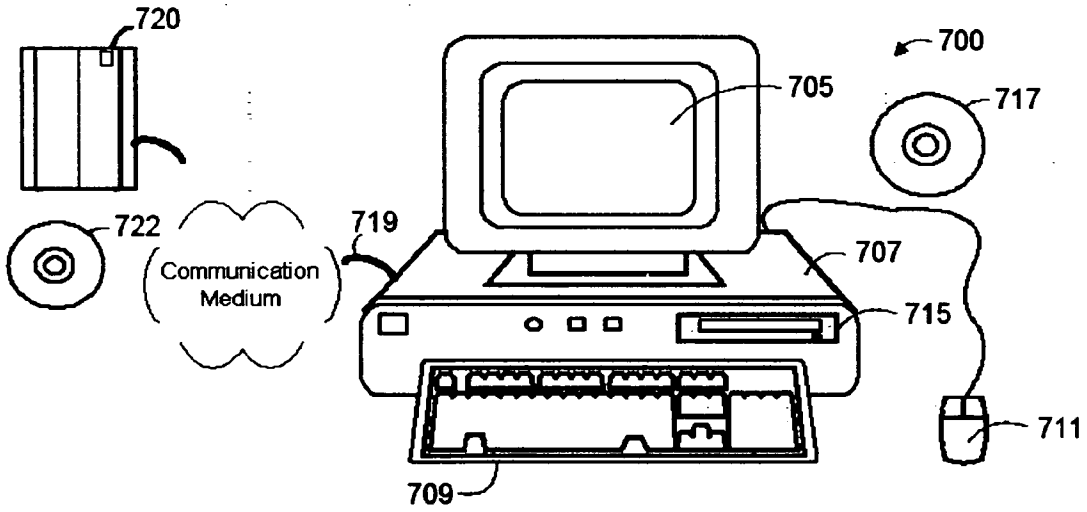

FIG. 11

Disease Classification	*Disease*
Cardiovascular Disease	Atherosclerosis; Unstable angina; Myocardial Infarction; Restenosis after angioplasty or other percutaneous intervention; Congestive Heart Failure; Myocarditis; Endocarditis; Endothelial Dysfunction; Cardiomyopathy
Endocrine Disease	Diabetes Mellitus I and II; Thyroiditis; Addisson's Disease
Infectious Disease	Hepatitis A, B, C, D, E; Malaria; Tuberculosis; HIV; Pneumocystis Carinii; Giardia; Toxoplasmosis; Lyme Disease; Rocky Mountain Spotted Fever; Cytomegalovirus; Epstein Barr Virus; Herpes Simplex Virus; Clostridium Dificile Colitis; Meningitis (all organisms); Pneumonia (all organisms); Urinary Tract Infection (all organisms); Infectious Diarrhea (all organisms)
Angiogenesis	Pathologic angiogenesis; Physiologic angiogenesis; Treatment induced angiogenesis
Inflammatory/Rheumatic Disease	Rheumatoid Arthritis; Systemic Lupus Erythematosis; Sjogrens Disease; CREST syndrome; Scleroderma; Ankylosing Spondylitis; Crohn's; Ulcerative Colitis; Primary Sclerosing Cholangitis; Appendicitis; Diverticulitis; Primary Biliary Sclerosis; Wegener's Granulomatosis; Polyarteritis nodosa; Whipple's Disease; Psoriasis; Microscopic Polyanngiitis; Takayasu's Disease; Kawasaki's Disease; Autoimmune hepatitis; Asthma; Churg-Strauss Disease; Beurger's Disease; Raynaud's Disease; Cholecystitis; Sarcoidosis; Asbestosis; Pneumoconioses
Transplant Rejection	Heart; Lung; Liver; Pancreas; Bowel; Bone Marrow; Stem Cell; Graft versus host disease; Transplant vasculopathy
Leukemia and Lymphoma	

FIG. 12. (TABLE 1)

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By: _____

QIPLG Attorney Docket No.: 313S.300800US Client Ref. No.: B04-030

PROVISIONAL PATENT APPLICATION

METHOD AND APPARATUS FOR PATCH-CLAMP ANALYSIS

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PATENT

METHOD AND APPARATUS FOR PATCH-CLAMP ANALYSIS

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FIELD OF THE INVENTION

[0002] The present invention relates to methods and/or system and/or apparatus involving patch-clamp analysis of cells and that can be adapted to other applications. In specific embodiments, the invention involves methods and/or system and/or apparatus involving various structures for manipulating objects, such as cells, in a fluidic medium and performing certail determinations thereof.

BACKGROUND OF THE INVENTION

[0003] The discussion of any work, publications, sales, or activity anywhere in this submission, including in any documents submitted with this application, shall not be taken as an admission that any such work constitutes prior art. The discussion of any activity, work, or publication herein is not an admission that such activity, work, or publication existed or was known in any particular jurisdiction.

[0004] The patch-clamp technique was described some 20 years ago to facilitate measurements and/or analysis of chemical and/or electrical properties at small regions of a cell membrane. Since then, patch clamp recording has had a profound impact on electrophysiology, playing a crucial role in the characterization of cellular ion channels. Traditionally, patch clamp recording is accomplished with a micromanipulator-positioned glass pipette under a microscope[1]. As illustrated in FIG. 1A a cell membrane patch is sucked into the glass pipette to form a high electrical resistance seal. Current that passes through the ion channels in either the membrane patch or the whole cell membrane is then recorded at different bias voltages. The properties of ion channels are central to nervous systems and often act as drug targets.

[0005] Despite improvements in the traditional patch clamp technique, it remains laborious and requires precisely pulled pipettes to be placed in the cell vicinity by a skillful operator using a micromanipulator under a microscope. Because of these requirements, the patch clamp technique has not been widely used in proteomics and drug discovery development, which demand high-throughput automated measurements. An automated patch clamp setup for high-throughput measurements using disposable devices would eliminate the prohibitive time investment of the traditional patch clamp, while maintaining its advantages over other measurements. Consequently, chip-based patch clamp devices have been proposed using silicon oxide coated nitride membranes[3], silicon elastomers[4], polyimide films[5], quartz[6] or glass[7] substrates. Recently, three dimensional structures more similar to patch pipettes have also been fabricated[8]. Chip-based devices developed to date generally use the planar geometry shown in FIG. 1B, where the patch pore is etched in a horizontal membrane dividing the top cell compartment from the recording electrode compartment.

[0006] Some more recent published patent applications that discuss various strategies related to patch-clamp analysis and related activities include the following U.S. applications, which are incorporated herein by reference to provide background.

1	20040005696	Substrate and method for measuring the electro-physiological properties of cell membranes
2	20030143720	High throughput functional genomics
3	20030139336	Interface patch clamping
4	20030138767	Liquid interface configurations for automated patch clamp recording
5	20030129581	Patch-clamping method and apparatus
6	20030065452	High throughput functional genomics
7	20030022268	Method and apparatus for patch-clamp measurements on cells
8	20020195337	Polymeric electrode for electrophysiological testing
9	20020182642	Biosensors and methods of using the same
10	20020064841	Planar patch clamp electrodes
11	20020045566	Selective maxi-K potassium channel openers functional under conditions of high intracellular calcium concentration, methods and uses thereof

[0007] Thus, some strategies have been discussed that utilize fabricated devices as part of a detecting device and/or system. Discussion of various of such strategies and related technology can be found in references cited in this submission. Furthermore, diagnosis and drug discovery is an essential tool in the health care industry. The role of diagnosis is expanding, particularly within the context of screening and prevention and use of patch-clamp analysis in some diagnosis could be expanded with improved systems.

[0008] Thus, in specific embodiments, the invention involves a novel patch-clamp system and/or associated methods. In specific embodiments, the invention avoids pipette fabrication and manipulation that make some earlier methods difficult.

Other References

1 B. Sackmann and E. Neher, *Single Channel Recording* (Plenum, New York, 1983).

2 J. Xu, X. B. Wang, B. Ensgn, M. Li, A. Guia, and J. Q. Xu, Drug Discovery Today **6,** 1278-1287 (2001).

3 N. Fertig, A. Tilke, R. H. Blick, J. P. Kotthaus, J. C. Behrends, and G. ten Bruggencate, Applied Physics Letters **77,** 1218-1220 (2000).

4 K. G. Klemic, J. F. Klemic, M. A. Reed, and F. J. Sigworth, Biosensors and Bioelectronics **17,** 597-604 (2002).

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SUMMARY

[0009] The present invention, in to specific embodiments, involves an integrated multiple patch-clamp array chip that in further embodiments utilizes lateral cell trapping junctions. In

specific example systems, the intersectional design of a microfluidic network provides multiple cell addressing and manipulation sites for efficient electrophysiological measurements at a number of patch sites. The patch pores consist of openings in the sidewall of a main fluidic channel, and a membrane patch is drawn into a smaller horizontal channel.

[0010] The device geometry according to specific embodiments of the invention not only minimizes capacitive coupling between the cell reservoir and the patch channel, but also allows for visual observation of membrane deformation. Device fabrication is based on micromolding of polydimethylsiloxane (PDMS), allowing for inexpensive mass production of disposable high-throughput biochips.

[0011] Thus, in specific embodiments, the present invention involves an integrated multiple patch-clamp array chip providing reliable lateral cell trapping junctions. In specific embodiments, the intersectional design of microfluidic network provides instantaneous multiple cell addressing. In specific embodiments, the geometry of this device not only minimizes capacitive coupling between the cell reservoir and the patch channel, but also allows for simultaneous optical and electrical characterization. Further, in specific embodiments, device geometry, together with the low dielectric constant of PDMS, results in very low capacitive coupling between the cell reservoir and the patch channel: $C_{predicted}$ = 0.4 fF and $C_{measured} \leq 1$ pF. The lateral design also allows for efficient multiplexing of patch measurements, exchange of intracellular electrolyte while the cell is attached to the patch pore, and optical observation of membrane deformation. This device has the potential for high throughput, low cost cell-based patch clamp measurements.

[0012] According to specific embodiments of the invention, aspects of the invention can be incorporated into an Integrated Microfluidic Patch-clamp Array Chip™ (IMPAC™) that provides a simple yet elegant way to trap multiple cells instantaneously by pneumatic controls and allows simultaneous electrical and optical characterizations. An IMPAC according to specific embodiments of the invention provides an ideal mechanism for high throughput screening (HTS) single cells analysis and drug discovery.

[0013] In further specific embodiments, the novel methods and devices according to specific embodiments of the invention can be used in various micrometer systems. Applications include BioMEMS, Lab on a chip, etc.

[0014] While example systems according to specific embodiments of the present invention is described herein as used for performing testing or characterizations of biological cells, it will be understood to those of skill in the art that a detector according to specific embodiments of the present invention can be used in a variety of applications for manipulating and assaying devices at a roughly cellular size. These applications include, but are not limited to: chemical systems; testing for contaminants in foodstuffs; detecting the presence of a desired substance or desired reaction, etc.

Other Features & Benefits

[0015] The invention and various specific aspects and embodiments will be better understood with reference to drawings and detailed descriptions provided in this submission. For purposes of clarity, this discussion refers to devices, methods, and concepts in terms of specific examples. However, the invention and aspects thereof may have applications to a variety of types of devices and systems. It is therefore intended that the invention not be limited except as provided in the attached claims and equivalents.

[0016] Furthermore, it is well known in the art that systems and methods such as described herein can include a variety of different components and different functions in a modular fashion. Different embodiments of the invention can include different mixtures of elements and functions and may group various functions as parts of various elements. For purposes of clarity, the invention is described in terms of systems that include many different innovative components and innovative combinations of innovative components and known components. No inference should be taken to limit the invention to combinations containing all of the innovative components listed in any illustrative embodiment in this specification.

[0017] In some of the drawings and detailed descriptions below, the present invention is described in terms of the important independent embodiment of a biologic assay and/or array system and components thereof. This should not be taken to limit the invention, which, using the teachings provided herein, can be applied to a number of other situations.

[0018] All references, publications, patents, and patent applications cited in this submission are hereby incorporated by reference in their entirety for all purposes.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates a comparison of some prior patch clamp setups: (a) Traditional patch clamp based on a glass micropipette. (b) On-chip planar patch clamp.

FIG. 2 illustrates a patch clamp setup according to specific embodiments of the invention showing (a) micro-array design with patch channels on the sides of a large central channel for cell delivery; a section containing two patch sides is shown; (b) top view of the patch clamp array device (optical microscope image) showing the central channel and 14 radial patch channels. The connectivity of the reference electrode and three of the patch electrodes is shown schematically and the small circle indicates one of the patch sites.

FIG. 3 illustrates operational details of an Integrated Microfluidic Patch-Clamp Array Chip (IMPAC) ™ according to specific embodiments of the invention. Some advantages of this overall design include easy cell trapping; easy optical characterizations; minimize RC; simple cell loading for multiple single cell analysis; high throughput and small drug usage; inexpensive and can create various designs of single cell analysis chip

FIG. 4 illustrates fabrication of the patch clamp micro-array according to an example specific embodiment of the invention. In this figure, the Si etch (a) is used to define the patch channels ($4\mu m \cdot 3.1\ \mu m$), while SU-8 photoresist (b) is used to define the large access channels (50 μm high). After PDMS is cured (c), the devices are detached and mechanically punched. The devices (d) and the glass substrate pre-coated with a thin PDMS layer (e) are treated with oxygen plasma. The devices are bonded to the thin PDMS (f). SEM images of overall device geometry before bonding (upside down) and a closeup of the patch pore after bonding are shown in panels (g) and (h). A SEM image of the mold is shown in a panel (i).

FIG. 5A and B illustrates a different view of an example integrated patch-clamp system according to specific embodiments of the invention.

FIG. 6 (a)–(c) illustrates three frames from a movie showing a HeLa cell being trapped by applying negative pressure (2 psi) to the patch channel. The third frame is magnified in order to show cell positioning on the patch pore. (d) Real time observation of the cell membrane deformation is shown.

FIG. 7 illustrates the current response to a 20 mV voltage pulse before (a) and after (b) cell trapping of an example device according to specific embodiments of the invention. From (a), the channel impedance is 14 MΩ, while in (b) the average seal resistance is 144 ± 3 MΩ.

FIG. 8 illustrates different shape of cell deformation at a clamp site according to specific embodiments of the invention providing for reduces leakage and improved sealing between a cell and device.

FIG. 9 illustrates aspects of a double channel-type patch clamp for example allowing for rapid change of intracellular and extracellular solutions according to alternative specific embodiments of the invention.

FIG. 10 illustrates aspects of a Disposable Polymer Concentric Clamp according to alternative specific embodiments of the invention.

FIG. 11 is a block diagram showing a representative example logic device in which various aspects of the present invention may be embodied.

FIG. 12 (Table 1) illustrates an example of diseases, conditions, or statuses that can evaluated or for which drugs or other therapies can be tested according to specific embodiments of the present invention.

DESCRIPTION OF SPECIFIC EMBODIMENTS

1. Definitions

[0019] The following definitions may be used to assist in understanding this submission. These terms, as well as terms as understood in the art should be used as a guide in understanding descriptions provided herein.

[0020] The term "microarray" or "high-density array" refers to a substrate or collection of substrates or surfaces bearing a plurality of array elements (*e.g.* discrete regions having particular moieties, *e.g.* proteins, nucleic acids, *etc.*, affixed thereto), where the array elements are typically present at a density of greater than about 10 elements/cm^2, preferably greater than about 100 elements /cm^2, more preferably greater than about 1000 elements /cm^2, and most preferably greater than about 10000 elements /cm^2, or 100000 elements /cm^2.

[0021] The term "microarray substrate" refers to a substrate suitable for the formation of a microarray comprising a plurality of array elements. The microarray substrate need not be used as

a component of a microarray. Thus, in certain embodiments, it is contemplated that such substrates can act as substrates for "macroscopic arrays" (*e.g.* dot blots),

[0022] A "substrate" is a, preferably solid, material suitable for the attachment of one or more molecules. Substrates can be formed of materials including, but not limited to glass, plastic, silicon, germanium, minerals (*e.g.* quartz), semiconducting materials (*e.g.* doped silicon, doped germanium, *etc.*), ceramics, metals, *etc.*

2. Device and Array Overview

[0023] To provide a context for understand specific embodiments of the present invention, consider an example integrated multiple patch-clamp array chip according to specific embodiments of the invention manufactured by utilizing lateral cell trapping junctions, as shown in FIG. 2. The geometry shown dramatically reduces the capacitive coupling between the cell reservoir and the patch channel, an important feature for low noise channel recording. Since the patch channels are in the horizontal plane, multiplexed parallel patch sites that are only tens of μm apart are possible. In the current design, the distance between patch sites is a few hundred μm. Channel binding drugs can therefore be administered in small volumes, while the effects on channel activity can be recorded in parallel at a number of patch sites. In one example fabrication, the whole device is fabricated using micromolding of polydimethylsiloxane (PDMS), a high-throughput, inexpensive procedure. FIG. 3 illustrates operational details of an Integrated Microfluidic Patch-Clamp Array Chip (IMPAC) ™ according to specific embodiments of the invention. Some advantages of this overall design include easy cell trapping; easy optical characterizations; minimize RC; simple cell loading for multiple single cell analysis; high throughput and small drug usage; inexpensive and can create various designs of single cell analysis chip

[0024] Example fabrication steps according to specific embodiments of the invention are presented in FIG. 4(a-f). In this example a silicon mold was prepared using surface micromachining techniques. In other examples, any other appropriate material and any other techniques for making a mold could be used.

[0025] In a micromachining example according to specific embodiments of the invention, first, 3.1 μm height patterns were made, defining the narrow patch channels using deep reactive ion etching (FIG. 4(a)). Second, 50 μm high patterns were added for wide connection regions

using SU-8 negative photoresist (FIG. 4(b)). After a base and a curing agent of PDMS were mixed (1:10), the liquid mixture was then poured onto the mold and cured at 80 °C for 1 hour.

[0026] SEM images of a fabricated example device are shown in FIG. 4 (g-h). In this example, it was observed that the top of the orifice is rounded. The rounding of the top of the orifice is a benefic mold fabrication artifact, and it was observed that the channel top is rounded next to the patch orifice in the mold (FIG. 4i). When the SU8 is selectively polymerized in order to create the large channels on top of the small patch channel defined in Si, light scattering near the Si surface results in a deviation from the intended vertical SU8 wall. The resulting rounded feature at the bottom of the SU8 wall (FIG. 4i, arrow A) is also present on top of the small Si wall (FIG. 4i, arrow B), resulting in rounding of the patch orifice top. This feature is reproducible as it is observed to be part of the mold geometry at every patch orifice.

[0027] For fluidic connections to outside tubing, 0.5 mm holes were punched mechanically into the cured and detached PDMS device. The device was subsequently bonded to a thin PDMS layer which was spin cast and then cured onto a glass substrate. Plastic tubes were connected to the reservoirs, via punched holes, to load both cells and electrolyte solutions and to apply suction to the patch channel.

3. Experimental Results

[0028] A human tumor cell line (HeLa), 12 to 17 μm in diameter, was used for seal resistance experiments. Before introducing the cells, the fluidic network was filled with phosphate buffered saline (PBS), taking care to expel all air bubbles. The electrical connection between the reference Ag/AgCl electrode in the main channels and the patch electrode in the lateral patch channel was confirmed by applying a 20 mV square pulse and recording the current response.

[0029] A typical channel current response is shown in FIG. 7 (a), indicating a channel resistance of 10-14 MΩ. This is comparable to the access resistance of traditional micropipettes, but can be reduced by altering the channel geometry. After dissociation by trypsin treatment, cells were suspended in PBS and injected into the main channel. Gentle pressure (1 psi) was applied to the patch channel while cells were loaded into the main fluidic channel in order to prevent contamination at the patch site. A cell can either be trapped randomly or selectively by controlling the flow through the main fluidic channel. A cell found within 100-200 μm of the patch channel opening can be trapped within a 1s time interval by applying 2 psi of negative

pressure to the patch channel. Right after trapping the cell, the negative pressure was removed and the cell was allowed to form a seal with the rim of the patch channel. The top down view allows for effective visualization of the membrane protrusion into the patch channel.

[0030] Patch resistance was recorded by applying a square voltage pulse of amplitude 20 mV and 50 ms duration. The current response was recorded using a standard patch-clamp amplifier (Dagan PC-ONE, Minnesota, USA) and low-pass filtered at 1 kHz. The current response presented contains no capacitance compensation. The resistance of the open patch channel was measured to be 14 ± 4 MΩ. The channel geometry (4μm · 3.1 μm · 200μm) and the conductivity of the electrolyte used (σ = 1 S/m) yield a calculated resistance of 17 MΩ, in reasonable agreement with the measurement. Capacitive coupling leads to a current spike when the bias voltage is first applied. Integrating spike currents gives an approximation to the charge stored in the capacitor by: $q = \int I\, dt$. Capacitance can then be calculated by using $C = q / V$. This capacitance measurement method yielded a capacitance of 10 ± 1 pF for connections between the device and the patch clamp amplifier input, but showed no further capacitance increase when the device itself was attached. We can conclude that the device capacitance is within the measurement error, or $C_{dev} \leq 1$ pF. Our calculations, using the device geometry and $\varepsilon_{PDMS} = 2.46$ [9], yielded a predicted device capacitance C_{dev} = 0.5 fF. By comparison, capacitances for micromachined patch clamp devices are 30pF for micronozzle devices[8] and 1pF for glass substrates[6], while micropipette capacitances are in the range of $2\text{pF} \leq C_{\text{pipette}} \leq 20$ pF.

Cell trapping

[0031] Cell trapping by suction is described above. The current response from the cell by a 20 mV/50ms current pulse is shown in FIG. 7 (b). The calculated sealing resistance after attaching the cell was 140 MΩ. Typical seal resistances were in the range of 140 ± 20 MΩ, while 200 MΩ was the highest seal resistance obtained. By applying positive pressure to the patch clamp channel, the trapped cell was expelled from the channel. As soon as the cell was expelled, the current response returned to that of the open channel. Subsequent cell trapping in the same channel resulted in lower seal resistance, presumably due to contamination at the opening of the patch channel.

[0032] Future improvements in PDMS surface treatment and patch pore geometry should lead to further increases in the seal resistance.

4. Packaging and Experimental Setup

[0033] According to specific embodiments of the present invention, an example sensor array consists of a number of sensor elements. In a particular example array, each sensor element is addressable via mechanisms that will be understood in the art and from the teachings herein. According to specific embodiments of the present invention devices, including electrical contacts and fluidic flow control can be assembled using techniques that will be understood from the art combined with the teachings of this submission and references herein.

5. Diagnostic and Drug Development Uses

[0034] As described above, following identification and validation of a assay for a particular cellular process, in specific embodiments such detectors are used in clinical or research settings, such as to predictively categorize subjects into disease-relevant classes. Devices according to the methods the invention can be utilized for a variety of purposes by researchers, physicians, healthcare workers, hospitals, laboratories, patients, companies and other institutions. For example, the devices can be applied to: diagnose disease; assess severity of disease; predict future occurrence of disease; predict future complications of disease; determine disease prognosis; evaluate the patient's risk; assess response to current drug therapy; assess response to current non-pharmacologic therapy; determine the most appropriate medication or treatment for the patient; and determine most appropriate additional diagnostic testing for the patient, among other clinically and epidemiologically relevant applications. Essentially any disease, condition, or status for which a cellular characteristic measurable using a patch clamp has been identified can be evaluated.

Web Site Embodiment

[0035] The methods of this invention can be implemented in a localized or distributed data environment. For example, in one embodiment featuring a localized computing environment, a patch clamp device according to specific embodiments of the present invention is configured linked to a computational device equipped with user input and output features. In a distributed environment, the methods can be implemented on a single computer, a computer with multiple processes or, alternatively, on multiple computers.

Kits

[0036] A device according to specific embodiments of the present invention is optionally provided to a user as a kit. Typically, a kit of the invention contains one or more patch claim

sensors constructed according to the methods described herein. Most often, the kit contains a diagnostic sensor packaged in a suitable container. The kit typically further comprises, one or more additional reagents, e.g., substrates, tubes and/or other accessories, reagents for collecting blood samples, buffers, e.g., erythrocyte lysis buffer, leukocyte lysis buffer, hybridization chambers, cover slips, etc., as well as a software package, e.g., including the statistical methods of the invention, e.g., as described above, and a password and/or account number for accessing the compiled database. The kit optionally further comprises an instruction set or user manual detailing preferred methods of using the kit components for sensing a substance of interest.

[0037] When used according to the instructions, the kit enables the user to identify disease specific substances cellular processes. The kit can also allow the user to access a central database server that receives and provides expression information to the user. Such information facilitates the discovery of additional diagnostic characteristics by the user. Additionally, or alternatively, the kit allows the user, e.g., a health care practitioner, clinical laboratory, or researcher, to determine the probability that an individual belongs to a clinically relevant class of subjects (diagnostic or otherwise). In HTS, a kit according to specific embodiments of the invention can allow a drug developer or clinician to determine cellular responses to one or more treatments or reagents, either for diagnostic or therapeutic purposes.

Embodiment in a Programmed Information Appliance

[0038] The invention may be embodied in whole or in part within the circuitry of an application specific integrated circuit (ASIC) or a programmable logic device (PLD). In such a case, the invention may be embodied in a computer understandable descriptor language, which may be used to create an ASIC, or PLD that operates as herein described.

Integrated Systems

[0039] Integrated systems for the collection and analysis of cellular and other data as well as for the compilation, storage and access of the databases of the invention, typically include a digital computer with software including an instruction set for sequence searching and/or analysis, and, optionally, one or more of high-throughput sample control software, image analysis software, data interpretation software, a robotic control armature for transferring solutions from a source to a destination (such as a detection device) operably linked to the digital computer, an input device (e.g., a computer keyboard) for entering subject data to the digital computer, or to control analysis operations or high throughput sample transfer by the robotic control armature. Optionally, the

integrated system further comprises an electronic signal generator and detection scanner for probing a microarray. The scanner can interface with analysis software to provide a measurement of the presence or intensity of the hybridized and/or bound suspected ligand.

[0040] Readily available computational hardware resources using standard operating systems can be employed and modified according to the teachings provided herein, e.g., a PC (Intel x86 or Pentium chip- compatible DOS,™ OS2,™ WINDOWS,™ WINDOWS NT,™ WINDOWS95,™ WINDOWS98,™ LINUX, or even Macintosh, Sun or PCs will suffice) for use in the integrated systems of the invention. Current art in software technology is adequate to allow implementation of the methods taught herein on a computer system. Thus, in specific embodiments, the present invention can comprise a set of logic instructions (either software, or hardware encoded instructions) for performing one or more of the methods as taught herein. For example, software for providing the described data and/or statistical analysis can be constructed by one of skill using a standard programming language such as Visual Basic, Fortran, Basic, Java, or the like. Such software can also be constructed utilizing a variety of statistical programming languages, toolkits, or libraries.

[0041] FIG. 11 is a block diagram showing a representative example logic device in which various aspects of the present invention may be embodied. FIG. 11 shows an information appliance (or digital device) 700 that may be understood as a logical apparatus that can read instructions from media 717 and/or network port 719, which can optionally be connected to server 720 having fixed media 722. Apparatus 700 can thereafter use those instructions to direct server or client logic, as understood in the art, to embody aspects of the invention. One type of logical apparatus that may embody the invention is a computer system as illustrated in 700, containing CPU 707, optional input devices 709 and 711, disk drives 715 and optional monitor 705. Fixed media 717, or fixed media 722 over port 719, may be used to program such a system and may represent a disk-type optical or magnetic media, magnetic tape, solid state dynamic or static memory, etc.. In specific embodiments, the invention may be embodied in whole or in part as software recorded on this fixed media. Communication port 719 may also be used to initially receive instructions that are used to program such a system and may represent any type of communication connection.

[0042] Various programming methods and algorithms, including genetic algorithms and neural networks, can be used to perform aspects of the data collection, correlation, and storage

functions, as well as other desirable functions, as described herein. In addition, digital or analog systems such as digital or analog computer systems can control a variety of other functions such as the display and/or control of input and output files. Software for performing the electrical analysis methods of the invention are also included in the computer systems of the invention.

[0043] Optionally, the integrated systems of the invention include an automated workstation. For example, such a workstation can prepare and analyze samples by performing a sequence of events including: preparing samples from a tissue or blood sample; exposing the samples to at least one array comprising all or part of a library of candidate probe molecules; and detecting the hybridization pattern by capacitance measurements. The hybridization pattern is digitized and recorded in the appropriate database.

[0044] Automated and/or semi-automated methods for solid and liquid phase high-throughput sample preparation and evaluation are available, and supported by commercially available devices. For example, robotic devices for preparation of cells. Alternatively, or in addition, robotic systems for liquid handling are available from a variety of sources, e.g., automated workstations like the automated synthesis apparatus developed by Takeda Chemical Industries, LTD. (Osaka, Japan) and many robotic systems utilizing robotic arms (Zymate II, Zymark Corporation, Hopkinton, Mass.; Orca, Beckman Coulter, Inc. (Fullerton, CA)) which mimic the manual operations performed by a scientist. Any of the above devices are suitable for use with the present invention, e.g., for high-throughput analysis of library components or subject samples. The nature and implementation of modifications to these devices (if any) so that they can operate as discussed herein will be apparent to persons skilled in the relevant art.

Other Embodiments

[0045] Although the present invention has been described in terms of various specific embodiments, it is not intended that the invention be limited to these embodiments. Modification within the spirit of the invention will be apparent to those skilled in the art.

[0046] It is understood that the examples and embodiments described herein are for illustrative purposes and that various modifications or changes in light thereof will be suggested by the teachings herein to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the claims.

[0047] All publications, patents, and patent applications cited herein or filed with this submission, including any references filed as part of an Information Disclosure Statement, are incorporated by reference in their entirety.